ON TRYPANOSOMA AND THEIR PRESENCE IN THE BLOOD OF BRISBANE RATS.

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(*Read before the Royal Society of Queensland. 3rd Sept.*, 1904)

During the past four years I have examined large numbers of rats of different species for plague, and occasionally in the systemic blood of some of the rats I detected the presence of those peculiarly interesting micro-parasites, the Trypanosomas. These organisms were first discovered by T. R. Lewis in India in 1879. He describes them as occurring in the blood of rats and hamsters which were apparently healthy. At first he thought they were spirilla, but on closer examination he found they possessed a distinct body outline, with at one end a flagellum. Lewis's original drawings of these organisms, which appear in the *Quarterly Journal of Microscopical Science* for that year, are somewhat primitive, compared with the same organisms seen with present day instruments. Lewis depicts them as having cylindrical or bitapering bodies, one end of which continued into a long lash-like thread. They were detected in twenty-nine per cent. of the species of rats Mus decumanus and Mus rufescens, and although from their movements and general appearance they resembled organisms of bacterial origin, Lewis considered they were more closely related to the Protozoa.

In 1880, Dr. G. Evans, of the Army Veterinary Department in India, discovered the presence of a Trypanosoma

parasite in the blood of horses, mules and camels which were suffering from a disease known as Surra, and which was extremely fatal to these animals in the Punjaub and British Brumah. In 1885, Veterinary Surgeon Steele further investigated the same disease and found the parasites in all cases, moreover both Evans and Steele proved by numerous experiments, that by means of subcetaneous inoculation and by the introduction into the stomach of blood containing the parasites the disease was transmitted to healthy animals. In 1882 Certes demonstrated the presence of Trypanosomas in body fluids of certain oysters. In 1883 micro-organisms similar to those found in the rat were observed by Mitrophanow as occurring in the blood of mud fish and the German carp.

In 1885 B. Danilewsky discovered Trypanosoma in the blood of birds, including ducks, geese, and fowls.

In 1886 Professor E. M. Crookshank very materially added to our knowledge of these organisms by investigating the morphology and life history of the Trypanosoma which he discovered in about 25 per cent. of the London sewer rats, and in the following year he succeeded in producing some excellent photo-micrographs, which clearly showed the presence of the flagellum, the dorsal undulating membrane, and the pulsating vacuole. In 1895 Dr. David Bruce announced the very important discovery that the tsetse fly disease, or Nagana, in Zululand was caused by a Trypanosoma. For over three years Bruce very closely studied the disease and conducted a series of interesting investigations, in which he not only brought to light many new morphological characters of the organism, but demonstrated its presence in the blood of horses, mules, asses, cattle, buffaloes, antelopes, camels, hyenas, and dogs; moreover from all these animals the organisms were transmissible to such experimental animals as the cat, rat, mouse, rabbit, hedgehog, donkey, bosch-bok, hybrid of zebra, guinea pig, goat, sheep, monkey, and weasel. He also proved that under natural conditions, the disease was transmitted from animal to animal solely by the tsetse fly (glossina moritans).

After Bruce's researches the subject of Trypanosomiasis was taken up and studied in various parts of the world, but

more particularly tropical countries, by many competent observers, notably Laveran, Koch, Lingard, Plimmer, Bradford, Theiler, Allen Smith, Voges, and Vsigburg.

The combined labours of these investigators have confirmed the researches of the more early workers on the subject, and have published much valuable information concerning the life history of the different species of Trypanosoma, technique of the methods for the detection, influence of sex, age and breed of the many species of animals that are subject to Trypanosomiasis, and also the questions of treatment of the disease and immunity.

In 1902 considerable interest was aroused in the medical profession by what may be taken to be a newly discovered disease in the human subject, for Dr. J. E. Dutton, of the School of Tropical Medicine in Liverpool, demonstrated the trypanosoma parasites in the blood of a man who had been living in the Gambia colony on the West Coast of Africa. Dutton mentions in his report that this patient was first seen by Dr. Forde in the Gambia colony in May, 1901, and in his blood he found small worm-like extremely active parasites, which was subsequently recognised by Dutton as trypanosoma. The symptoms presented by the patient were: Irregularly intermittent febrile attacks, the temperature remaining above normal (2 or 3 degrees) for a few days, and then falling below normal for a few days; the skin dry, with irregular patches of a congested or cyanosed character; puffy œdema of the face, and slightly around and above the ankles, respirations and pulse altered, being rapid and variable; heart sounds peculiarly muffled; urine and bowel excretions practically normal. In the later stages of the complaint both liver and spleen were found enlarged. Loss of weight and considerable debility, wasting, and lassitude were marked during the progress of the case. Dutton also found the parasite in the blood of a child, three years old, a native of the Gambia, but in whom no symptoms of illness were present. A little later Manson met with a patient in London whose symptoms were so suspicious that on examining his blood trypanosomas were detected, and thereby a significant advance in the clinical recognition of the disease was made.

Coming to a still more recent date. Aldo Castellani, in June, 1903, discovered an entirely new form of trypanosoma in the blood and cerebro-spinal fluid of patients suffering from sleeping sickness in Uganda on the shores of the lake Victoria Nyanza, in Central Africa.

So far in Queensland and possibly Australia Trypanosoma have only been demonstrated in the blood of rats, but I have proved their existence in at least three distinct species, viz., mus decumanus, the common brown rat; mus rattus, the old English black rat, which has very large, thin round ears, and a somewhat long, tapering tail; and mus Alexandrinus rufus, which is probably a hybrid between the brown and the black rat possessing all the morphological characters of the latter, but having a reddish-brown coat.

My observations are confirmatory of Crookshank's, in that rats having these parasites in their blood are apparently healthy.

Examination of Fresh and Stained Specimens.

If a drop of blood from a surra rat be examined under the microscope with $\frac{1}{6}$ objective it appears to quiver with life, and even with an oil immersion lense the parasites are extremely difficult to examine until their movement is arrested for a moment or they are imprisoned in the serum areas. As they are so actively motile they form very fascinating objects for the microscopist. A single organism will lash its flagellum in all directions as though endeavouring to free itself from its environment of red and white blood corpuscles. The body readily twists upon itself or from side to side with great activity. It can turn completely round with lightning rapidity, so that the flagellum will be lashing the blood cells for a moment in one direction, and then suddenly lash them in the opposite direction.

Sometimes it will spin round on its long axis and then at an incline on its short axis. Occasionally it appears as if attached by means of the spine-like process to a corpuscle, remaining stationary or lashing its flagellum. At first sight they appear to wriggle along either backwards or forwards, but the general mode of progression is by means of the fla-

gellum threading its way between the corpuscles, drawing the body of the organism after it, thus, as Crookshank states, the flagellum acts as a tractellum and not as a pulsellum. This feature of the movement of the flagellum in front drawing the body of the organism behind is almost a distinct peculiarity of such low types of animal life as the Protozoa.

All the trypanosoma are decidedly polymorphic, but as a rule they have slightly tapering bodies which terminate at one end with a stiff acutely pointed process, while the opposite end is provided with a long flagellum, which is a really a continuation of a delicate fin-like membrane attached to nearly two-thirds of the back of the organism. When carefully examined under a critical high power objective, the body substance is seen in parts to be distinctly granular and possessed of two or more highly refractive spherules; the one in the centre of the body being the nucleus, while at the posterior end there are usually two, one of which is the centrosome, and the other the pulsating vacuole.

The average size of the body of a trypanosoma is about 20 to 30 micro-millimetres long, and from 0.8 to 1 micro-millimetres broad; while the flagellum is about as long as the body, so that the total length of the organisms would be about 50 micro-millimetres, in fact many of them are in length from six to eight times the diameter of a red blood corpuscle, or roughly speaking the $\frac{1}{500}$ of an inch.

Nothwithstanding that trypanosoma may be detected in the blood in the living condition it is a very distinct advantage to be able to examine them after they have been fixed and stained and permanently mounted.

For this purpose I have been extremely successful in staining them with methylene blue, gentian violet, and Bismarck brown, in either of which watery solutions the coverglass smears after fixing with absolute alcohol should be left for at least from 12 to 24 hours; they are then washed in water and mounted in Xylol Balsam. One of the best and most permanent stains I find is carbol fuchsin; a little of this stain is placed in a watch glass, and the green metallic looking scum removed by the addition of two or three drops of alcohol. The coverglass smear is then floated, prepared

side down, and the whole gently heated over a spirit lamp or bunsen flame until the steam rises, when the green scum is about to make its re-appearance, remove the coverglass with a pair of forceps, wash in water and afterwards in very dilute alcohol; finally dry and mount in Xylol Balsam. In specimens so prepared all the morphological characters of the organisms are very clearly demonstrated. As to their permanency I have some preparations which I made in 1886 in London and which still show all the peculiar features of this organism.

The above stains are admirably adapted for photo-micrographic purposes, but for studying the structure of such delicate organisms I find the recently introduced blood staining methods of Romanowsky, Leishman and Jenner to give the most satisfactory results. By either of these methods, in which eosin and methylene blue dissolved in pure methyl alcohol are employed, different parts of the organism have an affinity for selecting in varying degrees one or other of the combined stains.

Of the three methods I prefer Romanowsky's: the blood to be examined is spread in a very thin film on a cover-glass, and allowed to dry spontaneously, which is sufficient to fix the specimen without passing through the flame, allow a few drops of stain to remain on the film for five minutes, then add an equal quantity of freshly distilled water, mixing gently for another three minutes, wash thoroughly in distilled water, dry in the air, and mount in xylol balsam.